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L14	VLP and L13	24	L14
L13	L12 and L10	84	L13
L12	chimeric	22969	L12
L11	dimer and L10	42	L11
L10	Gag and L9	149	L10
L9	receptor and L1	326	L9
L8	L1 and L4	0	L8
L7	L1 and L4L6	0	L7
L6	L4 and VLP	0	L6
L5	L4 and L2	6	L5
L4	Hodges R.in.	185	L4
L3	L1 and L2	14	L3
L2	Coiled adj coil	1548	L2
L1	(virus like particle)	793	L1

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L9	Hunt N.in.	35	L9
L8	VLP and L7	1	L8
L7	fusion and L6	41	L7
L6	receptor and L5	43	L6
L5	coil and L4	45	L5
L4	L3 and coiled	66	L4
L3	capsid adj protein	2490	L3
L2	coiled and L1	7	L2
L1	Hodges R.in.	185	L1

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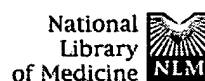
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☐**1:** Protein Eng 1996 Nov;9(11):1029-42   Related Articles, Links

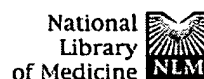## Engineering a de novo-designed coiled-coil heterodimerization domain off the rapid detection, purification and characterization of recombinantly expressed peptides and proteins.

Tripet B, Yu L, Bautista DL, Wong WY, Irvin RT, Hodges RS.

Department of Biochemistry, University of Alberta, Edmonton, Canada.

Using the techniques of genetic engineering and the principles of protein de novo design, we have developed a unique affinity matrix protein tag system as a rapid, convenient and sensitive method to detect, purify and characterize newly expressed recombinant peptides or proteins from cell extracts. The method utilizes two de novo-designed linear peptide sequences that can selectively dimerize to form the stable protein motif, the two-stranded alpha-helical coiled-coil. In this method, a recombinant bacterial expression vector pRLDE has been engineered so that one of the dimerization strands (E-coil) is expressed as a C-terminal fusion tag on newly expressed peptides or proteins, while the other (K-coil) is either biotin-labeled for detection in a Western blot-type format or immobilized on an insoluble silica support for selective dimerization affinity chromatography. Recombinantly expressed peptides from Escherichia coli containing the dimerization tag have been produced, detected and purified using this method. The recombinant peptides were easily and clearly identified using the biotin-labeled coil, while the single-step affinity purification results indicated the purity of the affinity purified expressed peptides to be > 95%, as assessed by reversed-phase chromatography. The stability of the dimerization domain also allows for the purified peptide to be left attached to the matrix, thus creating a new peptide-bound column that can be used to study peptide-protein or peptide-ligand interactions. Therefore this system offers a new alternative to existing peptide or protein fusion tags and demonstrates the utility of a de novo-designed system.

☐ **1:** Biochem Cell Biol 1996;74(2):133-54   Related Articles, Links

Boehringer Mannheim award lecture 1995. La conference Boehringer Mannheim 1995. De novo design of alpha-helical proteins: basic research to medical applications.

Hodges RS.

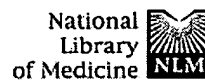Department of Biochemistry, University of Alberta, Edmonton, Canada.

The two-stranded alpha-helical coiled-coil is a universal dimerization domain used by nature in a diverse group of proteins. The simplicity of the coiled-coil structure makes it an ideal model system to use in understanding the fundamentals of protein folding and stability and in testing the principles of de novo design. The issues that must be addressed in the de novo design of coiled-coils for use in research and medical applications are (i) controlling parallel versus antiparallel orientation of the polypeptide chains, (ii) controlling the number of helical strands in the assembly (iii) maximizing stability of homodimers or heterodimers in the shortest possible chain length that may require the engineering of covalent constraints, and (iv) the ability to have selective heterodimerization without homodimerization, which requires a balancing of selectivity versus affinity of the dimerization strands. Examples of our initial inroads in using this de novo design motif in various applications include: heterodimer technology for the detection and purification of recombinant peptides and proteins; a universal dimerization domain for biosensors; a two-stage targeting and delivery system; and coiled-coils as templates for combinatorial helical libraries for basic research and drug discovery and as synthetic carrier molecules. The universality of this dimerization motif in nature suggests an endless number of possibilities for its use in de novo design, limited only by the creativity of peptide-protein engineers.

Publication Types:
- Biography
- Historical Article
- Lectures
- Review
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Personal Name as Subject:
- Hodges RS

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Use of a heterodimeric coiled-coil system for biosensor application and affinity purification.

Chao H, Bautista DL, Litowski J, Irvin RT, Hodges RS.

Protein Engineering Network of Centres of Excellence, University of Alberta, Edmonton, Canada.

The two-stranded alpha-helical coiled-coil is now recognized as one of nature's favorite ways of creating a dimerization motif. Based on the knowledge of protein folding studies and de novo design model systems, a novel heterodimeric coiled-coil protein was synthesized. The heterodimeric E/K coiled-coil was constructed with two distinct peptides (E and K) that will spontaneously associate into a full helical coiled-coil structure in solution. Equilibrium CD, NMR and real time biosensor kinetics experiments showed that the E/K coiled-coil is both structurally (deltaG(unfold)=11.3 kcal/mol) and kinetically (Kd approximately 1 nM) stable in solution at neutral pH. The engineered coiled-coil had been applied as a dimerization and capture domain for biosensor based applications and used in an expression/detection/affinity chromatography system. Specific test examples demonstrated the usefulness of the E/K heterodimeric system in these applications. The universality of coiled-coil as a dimerization motif in nature and our ability to design and synthesize these proteins suggest a wide variety of applications.
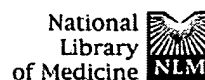
Publication Types:
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  o Review, Academic

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ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Coiled coils: new structures and new functions.

Lupas A.

Abteilung fur Molekulare Strukturbiologie, Max-Planck-Institut fur Biochemie, Martinsried, Germany. lupas@vms.biochem.mpg.de

Over the past five years, the structures of more than 20 proteins containing coiled-coil domains have been solved to high resolution. This has provided many new insights into the structure of coiled coils, their discontinuities, their relationship with other helical bundles and the problems connected with their prediction from protein sequences.

Publication Types:
  ○ Review
  ○ Review, Tutorial

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ERRATUM

Engineering a de novo designed coiled-coil heterodimerization domain for the rapid detection, purification and characterization of recombinantly expressed peptides and proteins

**Brian Tripet, Lei Yu, Daisy L.Bautista, Wah Y.Wong, Randall T.Irvin and Robert S.Hodges**

In Table II, mistakes were introduced into the sequences.
The correct Table II is given below:

Table II. Amino acid sequences of synthetic and recombinant peptides

Peptide name	Sequence[a]
	◄───────── Dimerization domain ─────────►
	gabcdefgabcdefgabcdefgabcdefgabcdef
K-coil	Ac-KVSALKEKVSALKEKVSALKEKVSALKEKVSALKEGGGnLC*-Amide
E-coil	NH$_2$-ALEGTEFGGGGGGGGGEVSALEKEVSALEKEVSALEKEVSALEKEVSALEKGGGGHHHHH-OH
PAK–pilin–E-coil	NH$_2$-ALEGTEF<u>KCTSDQDEQFIPKGCSK</u>FGGGGGGGGGEVSALEKEVSALEKEVSALEKEVSALEKEVSALEKGGGGHHHHH-OH
PAK–pilin(met)–E-coil	NH$_2$-ALEGTEFM<u>KCTSDQDEQFIPKGCSK</u>MKFGGGGGGGGGEVSALEKEVSALEKEVSALEKEVSALEKEVSALEKGGGGHHHHH-OH

[a]Residues involved in forming the heterodimeric two-stranded α-helical coiled-coil dimerization domain are indicated (← →). Positions of the heptad repeat are denoted by the letters abcdefg, where positions a and d (bold) represent the 3–4 hydrophobic repeat. The N-terminal amino group and the C-terminal carboxyl groups of the synthetically prepared K-coil peptide have been acetylated and amidated. The position of the cysteine used for biotinylation or column immobilization is indicated by the *. The 17 residues of *Pseudomonas aeruginosa* pilin protein, strain K (PAK–pilin), residues 128–144, are underlined.

Oxford University Press apologizes for the error.
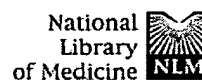
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Engineering a de novo-designed coiled-coil heterodimerization domain off the rapid detection, purification and characterization of recombinantly expressed peptides and proteins.
Protein Eng. 1996 Nov;9(11):1029-42.
PMID: 8961356 [PubMed - indexed for MEDLINE]

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☐ **2:** Tripet B, Yu L, Bautista DL, Wong WY, Irvin RT, Hodges RS.   Related Articles, Links

Engineering a de novo designed coiled-coil heterodimerization domain for the rapid detection, purification and characterization of recombinantly expressed peptides and proteins.
Protein Eng. 1997 Mar;10(3):299. No abstract available.
PMID: 9153081 [PubMed - indexed for MEDLINE]

☐ **3:** Chao H, Bautista DL, Litowski J, Irvin RT, Hodges RS.   Related Articles, Links

Use of a heterodimeric coiled-coil system for biosensor application and affinity purification.
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J Chromatogr B Biomed Sci Appl. 1998 Sep 11;715(1):307-29. Review.
PMID: 9792518 [PubMed - indexed for MEDLINE]

☐ **4:** Hodges RS.   Related Articles, Links

Boehringer Mannheim award lecture 1995. La conference Boehringer Mannheim 1995. De novo design of alpha-helical proteins: basic research to medical applications.
Biochem Cell Biol. 1996;74(2):133-54. Review.
PMID: 9213423 [PubMed - indexed for MEDLINE]

☐ **5:** Chong S, Montello GE, Zhang A, Cantor EJ, Liao W, Xu MQ, Benner J.   Related Articles, Links

Utilizing the C-terminal cleavage activity of a protein splicing element to purify recombinant proteins in a single chromatographic step.
Nucleic Acids Res. 1998 Nov 15;26(22):5109-15.
PMID: 9801307 [PubMed - indexed for MEDLINE]

☐ **6:** Schmidt TG, Skerra A.   Related Articles, Links

The random peptide library-assisted engineering of a C-terminal affinity peptide, useful for the detection and purification of a functional Ig Fv fragment.
Protein Eng. 1993 Jan;6(1):109-22.
PMID: 8433964 [PubMed - indexed for MEDLINE]